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(74) Agent: **WEBB, Cynthia**; P.O. Box 2189, 76121 Rehovot (IL).

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(54) Title: NOVEL HAPTOTACTIC PEPTIDES

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00, 38/08, 38/10, 39/00, 47/00, 48/00; C07K 4/00, 17/00; C12Q 1/16

US CL : 530/326, 327, 328, 333, 402; 514/2; 424/9.1, 9.322, 130.1, 278.1; 435/91.1, 35; 436/172, 504

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/326, 327, 328, 333, 402; 514/2; 424/9.1, 9.322, 130.1, 278.1; 435/91.1, 35; 436/172, 504

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WATALA, C. et al. Micro environmental changes in platelet membranes induced by the interaction of fibrinogen-derived peptide ligands with platelet integrins. Eur. J. Biochem. (1996) Vol. 235, 281-288. See entire document.	1 -2
X, P --- Y, P	US 6083902 (CEDERHOM-WILLIAMS) 4 July 2000, especially columns 15-16 and SEQ ID NOs. 27 and 28 (amino acid residues 416-435).	1 ---------- 2
X --- Y	CHUNG, D. W. et al. « Characterization of complementary deoxyribonucleic acid and demonic deoxyribonucleic acid for the b chain of human fibrinogen", Biochemistry (1983) Vol. 22, page 3244-3250. See polynucleotide sequence of Figure 3 (441-461).	1 ---------- 2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 May 2002 (02.05.2002)	17 JUL 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer<br~~Christopher S Low~~ Samuel W. Liu Telephone No. ~~703-305-9000~~ 703-306-3483

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL01/00057

~~Box I Observations where certain claims were found unsearchable~~ (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 3-25 and 29
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claims 3-25 unsearchable because there is no sequence listing and the CRF present in the instant international application. These claims are directed to the claimed SEQ ID Nos 1-12.

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN REGISTRY, CA, USPATFULL, BIOSIS, MEDLINE, EMBASE.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,599,790 A (ALTERI et al.) 04 February 1997, see entire document.	1-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer AVIS M. DAVENPORT Telephone No. (703) 308-0196

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NOVEL HAPTOTACTIC PEPTIDES

FIELD OF THE INVENTION

The present invention relates to novel cell attachment peptides, designated herein as haptotactic peptides, and in particular to peptides which are homologous to specific portions of the carboxy termini of fibrinogen chains that appear in other proteins, as well as to pharmaceutical compositions comprising such haptotactic peptides and potential uses for such compositions.

BACKGROUND OF THE INVENTION

Fibrinogen is the plasma protein responsible for blood clot formation. Normal fibrinogen is a complex of 2 sets of 3 chains (α,β and γ) (1-6). A variant of α fibrinogen (fib_{340}) fibrinogen with an extended α chain known as αE fibrinogen (fib_{420}) that constitutes about 1% of the total fibrinogen in adult humans has more recently been discovered but its unique function is not yet clear (7-10). Thus, the four types of fibrinogen chains, α, β, γ and αE, contain 610, 483, 411 and 866 amino acids, respectively (the numbering based on the Gene-bank database, accessible at info@ncbi.nlm.nih.gov).

Fibrinogen is not immunogenic within the same species, as attested by the use of pooled fibrin glue for clinical applications. Besides its hemostatic activity, it has been previously demonstrated that fibrin(ogen) elicits cell attachment (haptotactic) and migratory (chemotactic) responses with different cell types including mouse and human fibroblasts (MF and HF), bovine aortic endothelial (BAEC) and smooth muscle cells (SMC) (11,12).

The carboxy terminal sequences, i.e., the C-terminal 30-40 amino acids of the fibrinogen chains, are highly conserved between different species (13, 14). With the exception of the γ-chain C terminus (11,12), they have not been shown to relate to any hemostatic function of fibrinogen. A voluminous literature exists which describes the binding of fibrinogen (γ400-411) to platelets through the GPIIb/IIIa receptor and the aggregation activity of the new amino Bβ15-42 terminus that is exposed after release of fibrinopeptide B.

Fibrinogen fragment E was reported to exhibit angiogenic properties and to inhibit endothelial cell migration in a Boyden chamber chemotactic assay (19). The larger fragment D was reported to cause detachment of cultured endothelial cells from the extracellular matrix (ECM) substratum in a concentration and time dependent process (20).

Isolated constituent chains of fibrinogen (Aα1, Aα2 and Bβ) released upon activation of fibrinogen by thrombin were observed to stimulate fibroblast proliferation by 23-31% above controls, whereas isolated γ chain had no effect (22). Human polymorphonuclear leukocytes (PMN) were shown to bind to fibrin(ogen) coated surfaces via a type 3 (CD11b/CD18) complement receptor homologous to the GPIIb/IIIa receptor through a decamer of the γ chain carboxy terminus (LGGAKQAGDV). Vasoactive peptides were identified corresponding to residues 43-47 of the Bβ chain and 220-230 of the Aα chain (21).

The biological activities of a few other fibrinogen breakdown products have been investigated, but the cellular activity seemed to be widely variable (23).

Functional peptide sequences previously have been disclosed on the γ-chain, including sites involved in platelet binding (γ 400-411), leukocyte adhesion (γ 396-411),

factor XIII-crosslinking sites (γ 398, γ 407), a polymerization region (γ 374-395), and fibroblast adhesion region (γ 374-394). Thus, fibrinogen interactions with platelets and cells have been documented by a number of workers.

It has previously been disclosed by the present inventors (WO99/61041) that certain cell attachment effects of the intact fibrin(ogen) could be ascribed to small sequences at the carboxy termini of all the fibrinogen chains. Synthetic peptide fragments of the last 19-21 amino acids of carboxy termini of the α, β and γ chains of normal fibrinogen and of the αE chain (peptides termed Cα, Cβ, Cγ and CαE respectively), were tested. Only Cβ and CαE sequences induced significant haptotactic responses from various cultured cell types, mostly of mesenchymal origin, such as HF, BAEC and SMC, whereas the Cα and Cγ peptides did not exhibit significant haptotactic (cell attachment) activity. The active peptide Cβ was shown to be rapidly taken up by the cells in a non-saturatable manner. None of the disclosed peptides affected the rate of cell proliferation.

The identification of new haptotactic epitopes would have a number of applications, enabling more specific intervention in the wound healing process and the development of novel therapeutic compositions or devices. Furthermore, novel diagnostic tests to monitor cellular haptotactic responses could potentially be developed. Such peptides may have the ability to elicit haptotactic responses from cells, with no need to utilize the whole fibrinogen molecule and its attendant safety and regulatory issues.

Thus, there is a recognized need for, and it would be highly advantageous, to have peptides with specifically determined cellular effects, such as chemotactic or

haptotactic properties, which do not require the presence of the entirety of the fibrin(ogen), or the entirety of other proteins containing a homologous sequence.

SUMMARY OF THE INVENTION

It is an object of the present invention to identify and characterize haptotactic peptides with novel amino acid sequences that are homologous to known haptotactic peptides present within the carboxy termini of fibrinogen chains.

Haptotactic peptides are characterized in that they induce cell attachment to a surface to which such a peptide is covalently bound, inasmuch as the number of cells attached to such a surface will be at least 50% greater than the number of cells attached to the same surface absent the peptide. Preferably, the number of cells attached to such a surface will be at least 70% greater than the number of cells attached to the same surface absent the peptide. More preferably, the number of cells attached to such a surface will be at least double the number of cells attached to the same surface absent the peptide.

It is a first object to identify additional haptotactic peptides within or adjacent to the carboxy termini of fibrinogen chains. It is a further object of this invention to identify and characterize novel haptotactic peptides from additional proteins or polypeptides, containing at least one amino acid sequence homologous to such a fibrinopeptide. These novel haptotactic peptides are characterized in that they induce cell attachment to a surface to which such a peptide is covalently bound, inasmuch as the number of cells attached to such a surface will be at least 50% greater preferably 70% greater and more preferably double the number of cells attached to the same surface absent the peptide.

The degree of homology of the novel peptides to the fibrinopeptides will be at least 50%, preferably 60%, more preferably 70% and most preferably 80% or greater.

It is another object of the present invention to provide pharmaceutical compositions comprising as an active ingredient a novel haptotactic peptide according to the invention. It is another object of the present invention to provide medical implants or devices comprising as an active ingredient a novel haptotactic peptide according to the invention.

It is yet another object of the present invention to provide methods of using haptotactic peptides according to the invention in the treatment of a wound, disease or disorder comprising administering to an individual in need thereof a therapeutically effective amount of a haptotactic peptide according to the invention.

It is yet another object of the present invention to provide methods of using haptotactic peptides according to the invention in the treatment of a wound, disease or disorder comprising implanting into an individual in need thereof a medical implant or device comprising as an active ingredient a novel haptotactic peptide according to the invention.

It is another object of the present invention to provide pharmaceutical compositions comprising mixtures or combinations of haptotactic peptides according to the present invention. In this aspect the term combination may include both covalent attachments or non-covalent complexes or non-covalent mixtures. It is yet another object of the present invention to provide pharmaceutical compositions comprising at least one haptotactic peptide, further comprising at least one additional drug or biologically active agent. The additional drug or biological agent may be present in the composition as a non-covalent mixture or as a covalent conjugate with the haptotactic peptide.

It is still another object of the present invention to provide such haptotactic peptides useful for cell culture and cell separation. It is yet another object of the present

invention to provide such haptotactic peptides useful for fabricating novel cell-containing structures, including biomedical devices. It is yet another object of the present invention to provide such haptotactic peptides useful for coating natural or synthetic matrices. It is a further object of the present invention to provide such haptotactic peptides useful for accelerating the migration and attachment of cells to implants. It is yet another object of the present invention to provide a haptotactic peptide for attracting selected cell types into a biomedical device. It is yet another object of the present invention to provide such haptotactic peptides useful for targeting drug and biological factor uptake into different cell types.

These and other objects of the present invention are explained in greater detail in the description, Figures and claims below.

The novel peptide sequences of the present invention are homologous to certain known peptides of fibrin(ogen). They are derived either from hitherto undisclosed active fragments of fibrinogen or from certain other proteins or polypeptides containing homologous sequences, that retain certain desired properties of the entire molecule, such as cell adhesive effects, as defined above.

Within the scope of the present invention it is to be understood that the haptotactic peptides disclosed are preferred embodiments and intended to be construed as encompassing shorter active fragments thereof as well as homologs, derivatives and analogs, as defined hereinbelow.

Certain currently more preferred embodiments according to the invention include the following 19-21 mer peptides:

KTRWYSMKKTTMKIIPFNRL (peptide preCγ, SEQ ID NO. 1;)

KGPSYSLRSTTMMIRPLDF (peptide-C-ang1, SEQ ID NO. 2;)

KGSGYSLKATTMMIRPADF (peptide-C-ang2, SEQ ID NO. 3);

KGFEFSVPFTEMKLRPNFR (peptide-C-tenX, SEQ ID NO. 4), and

KGFYYSLKRPEMKIRRA (peptide-C-mfap, SEQ ID NO. 5),

Additional currently preferred embodiments include shorter sequences that were also determined to be haptotactic. The currently preferred specific sequences comprising 8-10 mer cell attachment peptides are:

KGSWYSMR (peptide-$C\beta_8$, SEQ ID NO:6); or

KGSWYSMRKM (peptide-$C\beta_{10}$, SEQ ID NO:7)

KTRWYSMKKT (peptide-Pre$C\gamma_{10}$, SEQ ID NO:8);

KGPSYSLR (peptide-C-ang1_8, (SEQ ID NO:9) and

KGFYYSLKRP (peptide-C-mfap$_{10}$, (SEQ ID NO:10).

The 19-21mer sequences as set forth in SEQ IDs # 1, 2, 3, 4 and 5 are equivalent to the C terminal amino acids of preCγ, C-ang1, C-ang2, C-tenX, and C-mfap, respectively.

The 8-10-mer sequences as set forth in SEQ IDs # 6, 7, 8, 9 and 10 are homologous to the first 8-10 amino acids sequences of the 19-21mer haptotactic Cβ and pre-Cγ, C-mfap and C-ang1, respectively.

Based on the high attachment activity of the synthetic 19-21mer peptides homologous to sequences in fibrinogen Cβ and preCγ chains as well as the other proteins with homologous sequences, a haptotactic consensus sequence called $HAPT_{15}$ (SEQ ID NO. 11) has been constructed comprising the amino acids:

$KGX_aX_bYSMRKX_cX_dMKIRP$;

wherein X denotes an amino acid, or may be absent thereby forming a direct bond.

Extensions at the N or C termini of this sequence are explicitly encompassed within the scope of the present invention. It should be noted that conservative replacements of the amino acid residues of this consensus sequence are also encompassed within the scope of the present invention, as is well known in the art.

Based on the activity of the synthetic 8-10 mer sequences a shorter haptotactic consensus sequence $HAPT_9$ epitope (SEQ ID NO. 12) was constructed:

KGX_aX_bYSMRK

wherein X denotes an amino acid or may be absent thereby forming a direct bond.

The $HAPT_{15}$ and $HAPT_9$ consensus sequences themselves, as well as analogs or derivatives comprising an additional spacer moiety or rearrangement for proper geometrical configuration, are also disclosed herein as haptotactic peptides of the present invention. It is intended to be understood that all known peptides encompassed within the generic formulae are explicitly excluded, including but not limited to the known haptotactic peptides of Cβ and CαE.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described by way of example only, with reference to the accompanying drawings, wherein:

Fig.1. Schematically depicts the principle underlying the haptotaxis assay utilizing peptide-coated Sepharose beads (SB). The CNBr activated SB (A) are reacted with the peptide to be tested resulting in SB-ligand (B). These are dropped onto a near confluent cell culture (C) and incubated. After a few hours the cells begin to attach the SB coated with haptotactic ligand (D). The fraction of SB-peptide attached to the cell

layer represents % haptotaxis. Non-coated SB or SB coated with non-reactive ligands (i.e. SB-albumin) do not attach.

Fig.2. Micrographs of SB-ligand reacting with endothelial cells (BAEC) after 2 days incubation. A: SB control, B: SB coated with preCγ, (SEQ ID NO:1) C: SB coated with Cangl (sequence ID#2) . By contrast to underivatized SB that do not attract cells, SB coated with reactive ligands become attached to the cell monolayer .

Fig. 3 Shows binding and internalization of dissolved 100μg/ml A: $C\beta^{FITC}$, B: $preC\gamma^{FITC}$ (SEQ ID NO:1) and C: Cmfap (SEQ ID No: 5) by HF as viewed by fluorescent microscopy after 1 hr incubation. Cells initially accumulated tagged peptides at the cell membrane and eventually became distributed within the cytoplasm, to the perinuclear area and into granular bodies with little penetration into the nucleus.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel peptides, which are homologous to haptotactic epitopes of fibrinogen, as well as to uses for these sequences in vivo as well as in vitro. For example, these peptide sequences have potential medical uses, including but not limited to therapeutic and diagnostic uses. The synthetic peptide sequences are homologous to regions of the fibrin(ogen) molecule, yet retain certain desired properties of the entire molecule, such as cell adhesive effects.

In particular, these cell attachment peptides comprise novel sequences homologous to 19-21 amino acids sequence of the carboxy termini of the β chain and αE chains of fibrinogen, which are now disclosed in other regions of fibrinogen as well as in other proteins.

The term "fibrin(ogen)" is known in the art and denotes either fibrinogen or fibrin or a mixture of fibrin and fibrinogen, and is referred to herein according to this definition. Hereinafter, the term "biologically active" refers to molecules, or complexes thereof, which are capable of eliciting an effect in a biological system. Hereinafter, the term "fragment" refers to a portion of a molecule or a complex thereof, in which the portion includes substantially less than the entirety of the molecule or the complex thereof.

The term "amino acid" refers to compounds which have an amino terminus and carboxy terminus, preferably in a 1,2- 1,3-, or 1,4- substitution pattern on a carbon backbone. α-Amino acids are most preferred, and include the 20 natural amino acids (which are L-amino acids except for glycine), which are found in proteins, the corresponding D-amino acids, the biosynthetically available amino acids which are not found in proteins (e.g., 4-hydroxy-proline, 5-hydroxy-lysine, citrulline, ornithine, canavanine, djenkolic acid, β-cyanolanine), and synthetically derived α-amino acids, such as amino-isobutyric acid, norleucine, norvaline, homocysteine and homoserine. β-Alanine and γ-amino butyric acid are examples of 1,3 and 1,4-amino acids, and many others are well known to the art. Statine-like isosteres (a dipeptide comprising two amino acids wherein the CONH linkage is replaced by a CHOH), hydroxyethylene isosteres (a dipeptide comprising two amino acids wherein the CONH linkage is replaced by a $CHOHCH_2$), reduced amide isosteres (a dipeptide comprising two amino acids wherein the CONH linkage is replaced by a CH_2NH linkage) and thioamide isosteres (a dipeptide comprising two amino acids wherein the CONH linkage is replaced by a CSNH linkage) are also useful residues for this invention.

As used herein "peptide" indicates a sequence of amino acids linked by peptide bonds. The peptide analogs of this invention comprise a sequence of amino acids of 7 to

24 amino acid residues, preferably 8 to 21 residues, each residue being characterized by having an amino and a carboxy terminus.

Hereinafter, the term "peptide" refers a sequence of amino acids, while the term "peptidomimetic" refers to analogues and mimetics having substantially similar or identical functionality to that of the haptotactic peptide which it is intended to mimic, including analogues having synthetic and natural sequences.

Hereinafter, the term "haptotactic peptide" refers to amino-acid sequences or analogues or derivatives or peptido-mimetics thereof, which are capable of eliciting attachment responses from cells, whereby the attachment of the cells in the presence of the haptotactic molecule is at least 50% greater than that in the absence thereof.

Hereinafter the term "epitope" refers to the active site on a complex molecule, which can react with antibodies or cell receptors. The term "epitope" is used herein, but is not limited to describing relatively short linear peptidic sequences on polypeptides or proteins (such as 8-10 amino acids in length) which can induce cell haptotaxis by interacting with cell attachment sites. Epitopes may also be formed by amino acid residues at sites which are not contiguous in the primary sequence of the polypeptide.

Hereinafter, the term "wound-healing cells" refers to those cells, which promote healing of a wound, including, but not limited to, fibroblasts, smooth muscle endothelial cells, osteoblasts and chondrocytes.

Based on the known (WO99/61041) activity of the CαE and its sequence homology to Cβ, it is now disclosed that we have identified and characterized novel haptotactic peptides. One novel haptotactic peptide, which is homologous to the Cβ 20-mer sequence, comprises the fragment adjacent to, i.e. just preceding, the C-terminal of the γ chain, termed herein preCγ (γ 366-386). We have further identified other proteins

that contain regions with significant homology to Cβ. Table 1 summarizes these proteins in sub-sets based on their biological function (Table 1; 1st column) including hemostasis (fibrinogen), modulators of angiogenesis (angiopoietins) (24-30), microfibril associated glyco-protein of the vasculature (microfibril associated protein 4) (31-34) and extracellular proteins of the tenascin family (35-37).

For example, angiopoietin 1 (ang1) and angiopoietin 2 (ang2) (MW ~130 kDa) (25-28) contain the haptotactic motif shared by fibrinogen Cβ and preCγ (the degree of homology having a statistical significance of $p<0.001$). These factors are secreted by cells to modulate vasculature formation in normal and cancer tissue. While ang1 serves as a stimulator of capillary development, ang2 is an inhibitor thereof. The receptors for these angiopoietins have been identified as the tyrosine kinase receptors Tie 1- and Tie-2 (26-30).

The family of tenascins, which contains a fibrinogen-like domain (34-37) also contain a sequence homologous to Cβ. Tenascins have been associated with the growth of neurons, but are ubiquitous and may serve other developmental functions, including binding to and modulating membrane sodium channels. Cell receptors identified to date for tenascins include integrins $\alpha_8\beta_1$ and $\alpha_9\beta_1$. Some tenascins are organized as hexamers.

Smith-Magenis syndrome (SMS) is a clinically recognizable multiple congenital anomaly/mental retardation syndrome associated with deletion of chromosome 17p11.2. The gene encoding a human microfibril-associated glycoprotein-4 (MFAP4) has been mapped to the SMS region that has a fibrinogen-like domain. A full-length cDNA corresponding to the MFAP4 gene contains a coding region of 255 amino acids. Deletion of the MFAP4 gene locus in SMS patients has been considered in the pathogenesis of this genetic disorder (34-37).

Table 1. Extended family of proteins containing a sequence homologous to the Cβ

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
Biol. Function	**Protein Name**	**Code name**	**17-21 mer peptide (synthesized)**																				**Position**	**P value**
Fibrinogen Clotting	β chain	Cβ	K	G	S	W	Y	S	M	R	K	M	S	M	K	T	R	P	F	F	P	Q	463-83	0
	αE chain	CαE	R	G	A	D	Y	S	L	K	A	V	R	M	K	T	R	P	L	V	T	Q	847-66	0.000001
	internal γ chain	preCγ	K	T	R	W	Y	S	M	K	K	T	T	M	K	T	I	P	E	N	R	L	T	366-86 / 411	0.0001
Vasculo-genesis	Angiopoietin-1	Cang1	K	G	P	S	Y	S	L	R	S	T	T	M	M	T	R	P	L	D	F			480-98	0.046
	Angiopoietin-2	Cang2	K	G	S	G	Y	S	L	K	A	T	T	M	M	I	R	P	A	D	F			478-96	0.0007
	Microfibril assoc. GP4	Cmfap	K	G	E	Y	Y	S	L	K	R	P	E	M	K	I	R	R	A					239-55	0.0006
Develop.	Tenascin-r (restrictin)	CtenR	K	G	H	E	F	S	I	P	F	V	E	M	K	M	R	P	Y	N	H	R	L	1320-40 /1358	0.01
	Tenascin-x	CtenX	K	G	F	E	F	S	V	P	F	T	E	M	K	L	R	P	R	N	F	R	S	641-61 /673	0.03
	Tenascin-c	CtenC	K	G	H	E	H	S	I	Q	F	A	E	M	K	L	R	P	S	N	F	R	N	2166-86 /2201	0.01

Full homology: Dark gray

Positive Homology: light gray

Homology is defined as meaning positional identity relative to the sequence of interest, as employed by the gene-bank data bases. Partial sequence identity (termed "positive homology") also adds to the score by defining certain amino acids as equivalent to one another (i.e. positive homology groupings are: S=T=N; R=K=Q; F=Y; V=M=L=I).

The novel haptotactic peptides (comprising homologous sequence variants of known haptotactic peptides) were synthesized as individual peptides, namely: Fibrinogen γ chain peptide, designated herein as preCγ, (SEQ ID NO. 1); Angiopoietin 1 peptide, designated herein as C-ang1, (SEQ ID NO. 2); angiopoietin 2 peptide, designated herein as C-ang2 (SEQ ID NO. 3); tenascin X peptide designated herein as C-tenX (SEQ ID NO. 4), and microfibril associated glycoprotein 4 peptide designated herein Cmfap (SEQ ID NO.5).

Active fragments of these cell attachment peptides are also now disclosed comprising 8-10 amino-acid long peptides (8-10mers) of the above described haptotactic peptides such as:

$C\beta_8$, (SEQ ID NO: 6); $C\beta_{10}$ (SEQ ID NO :7);

$PreC\gamma_{10}$, (SEQ ID NO: 8);

$C\text{-}ang1_8$, (SEQ ID NO: 9) and

$C\text{-}mpfa_{10}$, (SEQ ID NO: 10).

The sSequences of these peptides are given in Tables 2 and 3 below.

Table 2. Name, composition and codes of haptotactic 19-21mer peptides

Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Code
	NH_2																				COOH	
C β	K	G	S	W	Y	S	M	R	K	M	S	M	K	I	R	P	F	F	P	Q	Q	known peptide
C αE	R	G	A	D	Y	S	L	R	A	V	R	M	K	I	R	P	L	V	T	Q		known peptide
PreC γ	K	T	R	W	Y	S	M	K	K	T	T	M	K	I	I	P	F	N	R	L		SEQ ID NO. 1
C ang1	K	G	P	S	Y	S	L	R	S	T	T	M	M	I	R	P	L	D	F			SEQ ID NO. 2
C ang2	K	G	S	G	Y	S	L	K	A	T	T	M	M	I	R	P	A	D	F			SEQ ID NO. 3
C tenX	K	G	F	E	F	S	V	P	F	T	E	M	K	L	R	P	R	N	F	R		SEQ ID NO. 4
C mfap	K	G	F	Y	Y	S	L	K	R	P	E	M	K	I	R	R	A					SEQ ID NO.5

Table 3 shows the names, codes and sequences of 8-10mer peptides, which were synthesized, and which were haptotactic when tested with cultured cells.

Table 3. Name, composition and codes of haptotactic 8-10 mer peptides

Name	1	2	3	4	5	6	7	8	9	Code
Cβ 8 mer	K	G	S	W	Y	S	M	R		SEQ ID NO: 6
Cβ 10 mer	K	G	S	W	Y	S	M	R	K	M SEQ ID NO: 7
Pre Cγ 10 mer	K	T	R	W	Y	S	M	K	K	T SEQ ID NO: 8
Cang-1 10mer	K	G	P	S	Y	S	L	R	S	T SEQ ID NO: 9
C mfap 8 mer	K	G	F	Y	Y	S	L	K		SEQ ID NO: 10

Hereinafter, the term "haptotactic peptide" refers to peptides shown in Tables 1, 2 and 3, as well as to analogues, derivatives, or peptido-mimetics thereof, which are capable of eliciting attachment responses from cells.

Based on the high attachment activity of the synthetic 19-21mer peptides homologous to sequences in fibrinogen Cβ and preCγ chains as well as the other proteins with homologous sequences, a haptotactic consensus sequence called $HAPT_{15}$ (SEQ ID NO. 11) has been constructed comprising the amino acids:

$KGX_aX_bYSMRKX_cX_dMKIRP$;

wherein X denotes an amino acid, or may be absent thereby forming a direct bond. Extensions at the N or C termini of this sequence are explicitly encompassed within the scope of the present invention. It should be noted that conservative replacements of the amino acid residues of this consensus sequence are also encompassed within the scope of the present invention, as is well known in the art.

Based on the activity of the synthetic 8-10 mer sequences a shorter haptotactic consensus sequence $HAPT_9$ epitope (SEQ ID NO. 12) was constructed:

KGX_aX_bYSMRK

wherein X denotes an amino acid or may be absent thereby forming a direct bond.

The haptotactic peptides of the present invention are contemplated for many different uses, including but not limited to the treatment of a wound bed. Additional uses of the haptotactic peptides of the present invention include, but are not limited to, the separation of different types of cells from mixed cell cultures, the implantation of peptide-coated prosthetic devices, the identification and analysis of cell receptor mechanisms, the design of peptide-derivatized drugs to augment drug delivery and for diagnostic purposes. Furthermore, as explained in greater detail below, the haptotactic peptides of the present invention or their DNA or RNA sequences can also be used as tools for biological analysis and for further research and development.

These contemplated compositions, composites and uses of the haptotactic peptides of the present invention are outlined in the examples below and are intended as illustrations only and are not meant to be limiting in any way.

EXAMPLES

The present invention is drawn towards novel cell attachment epitopes and in particular to novel peptides which are homologous to regions of the carboxy termini of fibrinogen chains. Methods of using these peptidic sequences are also contemplated, including methods for the promotion of wound healing, for use as pharmaceutical compositions either per se or in conjunction with a medical device or implant, for the separation of cells from mixed populations, for the identification and analysis of cell receptor mechanisms, for use in augmenting drug delivery, prevention of restenosis and for diagnostic purposes.

The principles and operation of the invention, using peptidic amino acid sequences of fibrin and homologous sequences according to the present invention may be better understood with reference to the following non-limiting illustrative examples.

The peptides of the present invention were synthesized and tested in cell culture systems as described below in the section entitled "Experimental Procedure". The results are given in the section entitled "Results".

Essentially, specific peptides in Tables 2 and 3 were synthesized and covalently attached to Sepharose beads, to form SB-peptide (e.g., SB-preCγ, SB-C-ang1, or SB C-tenX). Fibrinogen was similarly covalently attached to Sepharose beads, to form SB-Fib. The SB-ligand combination was then incubated with cultured cells. The data as shown in the "Results" section, indicated that a family of peptides homologous to the fibrinogen β-chain carboxy termini appeared to be potent for cell binding, showing potency equivalent to that of the parent fibrinogen molecule.

The binding experiments with FITC-tagged peptides also indicated that the haptotactic peptides could self aggregate as well as bind to fibrinogen, fibrin and liposomes. From a biophysical perspective, these results strongly suggest that the hydrophobic C-termini of the β-chain and analogues found in the αE chain and the internal γ-chain, probably play a role in fibrin self-assembly during the various polymerization interactions it undergoes following thrombin activation.

The peptides of the above invention are significantly homologous to one another. From the perspective of fibrinogen biology, these sequences are highly conserved. Based on the lack of immunogenicity of fibrinogen itself, these haptotactic fibrino-peptides are probably non-immunogenic, and advantageously are therefore not expected to elicit immune responses. Structure/Function studies were performed to identify smaller active regions of the

haptotactic peptides homologues to the fibrinogen Cβ. Selected modifications of the 19-21mer peptides covalently bound to Sepharose beads were carried out and their haptotactic activity evaluated.

The tools and techniques arising from these haptotactic peptides will find application in diverse fields associated with cell manipulation, wound healing, targeted drug delivery and tissue engineering.

Experimental Procedures

Preparation of Peptides

Synthesis of custom made C-terminal peptides: The peptides sequences presented in Tables 2 and 3 were synthesized using standard procedures, by commercial laboratories (Novatide Ltd., Haifa, Israel; SynPep Labs, California ,US; New York Blood Center Microchemistry Lab, New York, US). The experiments employed peptides that were >85-95% pure as determined by HPLC/mass-spectrometry.

Covalent coupling of peptides or proteins to Sepharose beads: Peptides, fibrinogen and other proteins were covalently bound to CNBr-activated Sepharose 4B beads (Pharmacia, Piscataway, NJ) in a procedure previously used to bind fibrinogen, thrombin and BSA (15,16). Concentrations of peptides bound to SB in different preparations were in the range of 2-7 μM. SB coated with either BSA, fibrinogen, fibronectin or thrombin were similarly prepared. The coated SB were stored in saline at 4°C with 0.1 % azide. Before testing with cell cultures, the beads were washed 3-5 times in sterile saline to remove all traces of azide.

SB Haptotaxis assay: The attachment of SB-ligand to cells in nearly confluent cultures was measured as previously described (15,16). Essentially, about 20 - 150 μl of suspended (50% v/v) SB-peptide or SB-protein were added to 6 - 24 well plates with near confluent cell cultures and dispersed by gentle shaking for 1 min. The plates were then incubated for up to

4 days. At different time points, the number of SB tethered to cells was counted with an inverted phase microscope. Typically, approximately 300 SB (but not less then 200) were counted in each well, and the ratio of the number of SB attached to the cells in each well, was calculated relative to the total number of SB. Only SB coated with haptotactic materials became attached to the cell layer, ultimately to be engulfed by cells and tethered to the plate. Without a ligand or coated with a neutral molecule such as BSA (control), none of the SB became attached to cells on the plate.

Percent SB attached to the cell surface at different time intervals provided a quantitative assay of the degree and the kinetics of the haptotactic response. At least 3 wells were measured for each variant and each experiment was repeated at least 3 times.

Monitoring cell number with the MTS assay: The MTS colorimetric assay (CellTitre 96 Aqueous Assay by Promega) was used to assay cell proliferation with peptide levels ranging up to 100 μg/ml and to evaluate the number of viable cells obtained in the adhesion assays. The MTS assay is based on dehydrogenate conversion of MTS by viable cells to colored tetrazolium salt and performed in 96 well plates, as previously described (15,16). The optical density (OD) of the dye was measured at 492 nm by a computerized microplate reader (Anthos HT-II, Salzburg). The OD of the dye was calibrated to correlate linearly with the cell number. The plating density and incubation conditions were optimized for each cell type.

Fluorescence microscopy and confocal laser fluorescence microscopy: Light and fluorescent microscopy were carried out using an Olympus system. Confocal laser microscopy was done with a computerized Zeiss Confocal Axiomate microscope (LSM410) with multiple excitation wavelengths. For examination of cell interaction with FITC-peptides, the cells were grown on glass coverslips to near confluence, then incubated with 10 μg/ml FITC-

tagged peptides at room temperature. At different time points, the cells were washed and fixed in 0.5% buffered glutaraldehyde. Coverslips with the cells were placed on a microscope slide with PBS-glycerol 80% with 2% DABCO and examined. The representative fields of cells were visualized by phase contrast Numarski optics. Fluorescence intensity at the FITC wavelength (excitation 488nm, emission 515nm) and scans were stored in the computer for further image reconstruction

Peptide binding to fibrin(ogen):

FITC-tagged peptides (10 μg/ml) were mixed with either SB-Fib, SB-peptide incubated for 1 hour at ambient temperature, and visualized by confocal fluorescent microscopy. Alternately, 100 μl of fibrin clot (2.4 mg/mL) was formed from fibrinogen and thrombin. After clot formation, 100 μl FITC-tagged peptides (10 μg/ml) were layered onto the clot, incubated for 1 hour at ambient temperature. The clot was washed with Tris buffer and visualized by confocal fluorescent microscopy.

Structure/Function tests

Some structure function tests were carried out by measuring the haptotactic response of a given cell to SB-peptide before and after treating the SB-ligand with either trypsin, or oxidation conditions or after undergoing acetylating reaction to acetylate free amines. Based on the results with such treatments with 19-21mer peptides, smaller 8-10mer peptides were synthesized, coupled to SB and tested for haptotactic activity, according to the methods described above.

Monitoring peptide uptake by cells by Fluorescence Microscopy

The cells examined were grown in 6-well plates on cover slips to reach near confluence. At the time of examination, the cover slips were inverted and put on a microscope slide supported by 2 thin spacers so that a thin gap (~2 mm) was left between the cells on the coverslip and the slide. This was filled with culture medium. To follow the uptake, 10 μg/mL FITC-labeled peptide was added into the culture medium in the gap. At different time points, medium was replaced with fresh medium and the fluorescence was viewed and photographed, using an Olympus fluorescent microscope system.

Coupling haptotactic peptide to Cellulose sponge and testing for cellularization:

Dissolve peptide in minimum DMSO, make <0.1 mM in phosphate buffer. Mix 1 mL of DSS (disuccinimidyl subarate) in DMSO (2.5 mM) in phosphate buffer and incubate with sponge for 30 min. at room temperature. Stop reaction by adding 1 mL Tris-saline buffer.

Pieces of peptide-coated sponge or untreated control were mixed with trypsinized fibroblasts (HF) or other cell types in cell culture medium and incubated. After 3 -21 days incubation, sponge samples were removed, fixed with 95% alcohol and 0.1 mM propidium iodide (PI) was added to stain the cell nuclei. The sample was rinsed and examined by confocal fluorescent microscopy.

Results

Example 1:

Summary of Haptotactic Effect of 19-21 mer peptides with fibroblasts (HF), endothelial cells (BAEC) and smooth muscle cells (SMC).

The % Haptotaxis was obtained by monitoring the attachment of 19-21mer peptide-coated Sepharose beads (SB-peptide) onto a near confluent cell layer. Periodically (i.e. 24 hr), the fraction of SB-peptide bound to the cell layer was counted out of a field of 200 or 300 SB

total. The results demonstrate that the peptides SEQ ID 1-5 are haptotactic as they can render an otherwise neutral SB attractive to cells at levels equivalent to fibrinogen.

Table 5. Haptotactic 19-21 mers

				% Haptotaxis 24hr		
Source Protein	**Code**	**SB-ligand**	**Pep. Code**	**EC**	**HF**	**SMC**
SB only (control)		**None**		**0**	**0**	**0**
Fibrinogen (control)		**Fib**		**100**	**97**	**99**
Fib α chain (control)	**7**	**Cα**	None	**0**	**0**	**0**
Fib β chain	**9**	**Cβ**	known peptide	**94**	**98**	**93**
Fib αE chain	**71**	**CαE**	known peptide	**77**	**77**	**4**
Fib γ chain	**70A**	**preCγ**	SEQ ID NO. 1	**99**	**98**	**95**
Fib γ chain	**70**	**Cγ**	None	**30**	**0**	**0**
Angiopoietin-1		**C-ang1**	SEQ ID NO. 2	**81**	**63**	**67**
Angiopoietin-2		**C-ang2**	SEQ ID NO. 3	**77**	**91**	**96**
Tenascin X		**C-tenX**	SEQ ID NO. 4	**41**	**94**	**100**
Microfibril assoc.protein		**C-mfpa**	SEQ ID NO. 5	**76**	**95**	**100**

Example 2:

Structure/Function tests of 19-21mer peptides

In order to identify smaller active regions of the haptotactic peptides homologues to the fibrinogen Cβ, selected modifications of the 19-21mer peptides covalently bound to sepharose beads were carried out and their residual haptotactic activity evaluated. Thus, it was noted that trypsin significantly reduced haptotactic activity of the Cβ but not the C-ang1. Acetylation of K and R moieties in Cβ reduced % haptotactic response of SMC and HF, but did not affect the responsiveness of EC. Oxidation of the M group particularly reduced the attractiveness of the peptides for HF. As only Cβ has an internal lysine (K) site capable of being digested by trypsin, and considering the lack of activity of the shorter Cβ12-21 (not

shown here), this indicates that the first 8-10 amino acids might be adequate for a minimal haptotactic epitope.

Table 6. Structure/function tests of SB-peptide with cells

		% Haptotaxis																							
SB-ligand	**Treat**	**EC**	**SMC**	**HF**	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
SB-Cβ	none	100	91	90	K	G	S	W	Y	S	M	R	K	M	S	M	K	I	R	P	F	F	P	Q	Q
	trypsin	0	0	10									•												
	acetyl	100	60	12								•	•				•		•						
	oxidize	100	80	40							•			•		•									
SB-C-ang1	none	100	50	3	K	G	P	S	Y	S	L	R	S	T	T	M	M	I	R	P	L	D	F		
	trypsin	90	40	0																					
	acetyl	100	95	0								•							•						
	oxidize	100	92	3												•	•								
SB-preCγ	none	100	100	60	K	T	R	W	Y	S	M	K	K	T	T	M	K	I	I	P	F	N	R		
	trypsin	0	0	0								•	•				•								
	acetyl	100	100	90			•					•	•				•		•						
	oxidize	100	93	3							•					•									

• denotes amino acid modified by treatment

These data suggested that the lysine (K) at position 9 was important for the haptotactic activity of Cβ, and further suggested that the sequences 1-10 might be critical to the haptotactic activity of the peptides. Based on these results (summarized in Table 6, a number of 10-mer peptides were synthesized and tested for haptotactic activity (see Table 7).

Example 3:

Summary of haptotactic effects of 8-10-mer peptides with fibroblasts (HF), endothelial cells (BAEC) and smooth muscle cells (SMC).

% Haptotaxis was monitored by following up the attachment of 10-mer peptide-coated Sepharose beads (SB-peptide) onto a near confluent cell layer (Figures 1and 2). Periodically,

the fraction of SB-peptide bound to the cell layer was counted out of a field of 200 or 300 SB total.

Table 7. Haptotactic 8-10 mers				% Haptotaxis 24hrs		
Source Protein		**SB-ligand**	**Peptide Code**	**EC**	**HF**	**SMC**
None (control)		None (control)		0	0	0
Fib β chain	RG-1	Cβ 8 mer	SEQ ID NO: 6	88	79	93
Fib γ chain		Pre Cγ 10 mer	SEQ ID NO: 8	100	100	100
Angiopoietin-1	RG-2	C-ang1 8 mer	SEQ ID NO: 9	75	45	4
Microfib. ass. prot.		C mfap 10 mer	SEQ ID NO: 10	100	99	100
Tenascin		Ten X 10mer	SEQ ID NO: 13	1	0	0

The results demonstrate that the 8-10mer peptides of SEQ ID 6-10 are indeed haptotactic as they can render an otherwise neutral SB attractive to cells.

Example 4:

Effect of haptotactic peptides on Cell Proliferation

To test whether the haptotactic peptides modulate cell proliferation, cells were incubated with a range of 1-50 uM concentrations of the peptides of interest for 3-4 days then the number of viable cells was determined with the MTS colorimetric assay. None of the peptides of SEQ ID 1-10 affected the rate of proliferation of HF, BAEC or SMC relative to untreated controls.

Example 5:

Uptake of FITC- Cβ and FITC- preCγ by cells by fluorescence microscopy

Exposure of cultured human fibroblast cells to a solution of 10 μM FITC peptide FITC- Cβ or preCγ (sequence ID #1) resulted in uptake into the cell cytoplasm, as shown by

fluorescence microscopy (Figure 3). After a longer exposure of more than 1 hour or with fixed cells, accumulation of the FITC-peptide in the cytoplasm and around the nucleus was clearly observed (data not shown). In most cases, the fluorescence became concentrated in discrete cytoplasmic vesicles.

These haptotactic peptides could be used to increase the cellularization of implants or to induce a better cellular contact with the implant. For example a peptide coated sponge implanted into bone tissue could induce osteogenic cells to migrate into and attach to the sponge and create improved new bone matrix at the site of the implant.

In another use, an electronic signaling or monitoring device coated with haptotactic peptides would exhibit improved binding to the cells within the implant area and be better incorporated into the tissue, thereby allowing its electronic functionality to be more efficient.

Polynucleotide sequences that encode for the amino acid sequences of the haptotactic peptides can be used to generate the peptides in genetically modified cells as is well known in the art. The DNA and RNA sequences can also be used for medical or diagnostic purposes. For example, one could monitor the mRNA sequences which encode for the haptotactic peptides to determine if those sequences are being biosynthesized by the cells or tissue being examined or if their synthesis is increased or decreased as a result of a therapeutic treatment or drug dosage..

Example 6:

Binding of FITC-tagged peptides to SB-Fib, SB-peptide or liposomes:

FITC-tagged peptides (10 μg/ml) were mixed with either SB-Fib, SB-Cb, incubated for 1 hour at ambient temperature, and visualized by confocal fluorescent microscopy. Similarly, 100 uL fibrin clot (2.4 mg/mL) was formed from fibrinogen and thrombin. After clot formation, 100 uL FITC-tagged peptides (10 μg/ml) were layered onto the clot , incubated

for 1 hour at ambient temperature. The clot was washed with Tris buffer and visualized by confocal fluorescent microscopy. Fluorecsent micrographs reveal that the haptotactic fibrino-peptides bind to fibrinogen and to itself (i.e. SB-peptide). The interactions of haptotactic peptides with liposomes indicate that these peptides can bind to hydrophobic cell membranes and possibly to hydrophobic regions of large molecules.

Without wishing to be limited by a single mechanism, functional cell attachment features of fibrinogen chains and homologues of fibrinogen chain, as in Tables 2 and 3 are critical to the normal development and wound healing of all species. Peptide analogues of those in Tables 2 and 3 could be synthesized with non-natural synthetic amino acids or with D-amino acids, which would also provide a means of modulating the rate of peptide degradation within the cell and thereby prolong their biological lifetime, or create more selectivity to different cell types.

EXAMPLE 7

Coating of matrices with haptotactic peptides increases cell attachment in-vivo as well as in-vitro

1. Cell culture model: A polymeric sponge containing free carboxy groups was covalently coated with haptotactic peptide according to known methods (38) as follows: Cβ peptide was coupled to the matrix by employing a water soluble carbodiimide reagent 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (MW 191.7, Pierce Co) as follows: Matrix (100 mg) suspended in 2ml conjugation buffer (0.1 M MES (2-[N-morpholinoethane sulfonic acid), pH 4.5-5). FITC-Cβ peptide (100 µl, 2 mg/ml) was added and the mixture stirred on an orbital shaker. EDC (2 mg) was added and the entire mixture was shaken at ambient temperature for 2 hours The reaction was stopped by adding 100 µl Tris/saline buffer and the matrix isolated. On the basis of the residual OD 280 of the supernatant, more than

70% of the FITC-peptide became coupled to the matrix to form peptide-matrix sponge.

A control polymeric sponge and Cβ-matrix sponge was incubated with trypsinized fibroblasts for over 10 days. Samples of sponge were removed at specified intervals (days 3 and 21), and were fixed and stained with propidium iodide to visualize cell nuclei. Confocal fluorescence micrographs show that relative to the untreated control sponge, the Cβ-treated sponge showed higher cellularization, namely an increase of cell content by more than 50% within 3 days, and the difference increased over a 21 day incubation period, where more than doubling of the the cell number was recorded. Similar results were obtained with other haptotactic peptides relative to untreated controls.

2. Animal model: Implant control sponge or Cβ -coated sponge under the skin of the back of rats and close the wounds. After 4.5 and 8 weeks, the animals were sacrificed and the implant areas examined histologically. In sets of control sponges, one could observe cells accumulating on the edges of the sponge and penetrating into the sponge inter-fibrous spaces. The cells form extracellular matrix with collagen deposition and granulation tissue, including the presence of giant cells and granulocytes and some inflammatory driven leukocytes. After 4.5 weeks, the sponges coated with Cβ peptide showed significantly increased cellularity consisting of both fibroblasts and leukocytes and formation of more granulation tissue, relative to the control.

EXAMPLE 8
Polynucleotide sequences encoding haptotactic peptides

Polynucleotide sequences that encode for the amino acid sequences of the haptotactic peptides can be used to generate the peptides in genetically modified cells as is well known in the art. The DNA and RNA sequences can also be used for medical or diagnostic purposes.

Without wishing to be limited, two examples of DNA sequences that encode for the amino acid sequences of the haptotactic peptides are as follows:

Cβ
DNA
...aaggggtcatggtactcaatgaggaagatgagtatgaagatcaggcccttcttcccaca
gcaa tag..
K G S W Y S M R K M S M K I R P F F P Q Q

Pre Cγ
DNA
...aaaacccggtggtattccatgaagaaaaccactatgaagataatcccattcaacagact
caca...
K T R W Y S M K K T T M K I I P F N R L T

The amino acids of the haptotactic peptides can be encoded by other variant DNA sequences. The DNA and RNA sequences that code for the amino acids of the haptotactic peptides can be used for medical as well as diagnostic purposes.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and other applications of the invention may be made. The scope of the invention is not intended to be defined by the particular exemplifications used for illustrative purposes herein, but rather by the claims which follow.

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CLAIMS

1. A haptotactic peptide with amino acid sequence that is at least 50% homologous to a known peptide present within the carboxy termini of fibrinogen chains selected from the group consisting of Cβ and CαE, and characterized in that it induces cell attachment to a surface to which the haptotactic peptide is covalently bound, inasmuch as the number of cells attached to such a surface will be at least 50% greater than the number of cells attached to the same surface absent said peptide.

2. The haptotactic peptide according to claim 1 wherein the sequence is at least 70% homologous to a known peptide present within the carboxy termini of fibrinogen chains.

3. The haptotactic peptide of claim 1, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1-5 and analogues, derivatives, homologues or fragments thereof, characterized in that it induces cell attachment to a surface to which the haptotactic peptide is covalently bound, inasmuch as the number of cells attached to such a surface will be at least 50% greater than the number of cells attached to the same surface absent said peptide.

4. A haptotactic peptide, comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:6-10 and analogues, derivatives or homologues thereof, characterized in that it induces cell attachment to a surface to which the haptotactic peptide is covalently bound, inasmuch as the number of cells attached to such a surface will be at least 50% greater than the number of cells attached to the same surface absent said peptide.

5. A haptotactic peptide, comprising an amino acid sequence derived from a consensus sequence selected from the group consisting of SEQ ID NOs:11-12 and an analogue or functionally equivalent amino acid sequence thereof, and characterized in that it induces cell attachment to a surface to which the haptotactic peptide is covalently bound, inasmuch as the number of cells attached to such a surface will be at least 50% greater than the number of cells attached to the same surface absent said peptide.

6. A pharmaceutical composition, comprising as an active ingredient a haptotactic peptide having a sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO 10, SEQ ID NO. 11, SEQ ID NO.12 and analogues, derivatives, fragments and homologues thereof.

7. The pharmaceutical composition of claim 6, further comprising a pharmaceutically acceptable carrier.

8. The pharmaceutical composition of claim 7, further comprising at least one additional drug or biological agent.

9. The pharmaceutical composition of claim 8, wherein said haptotactic peptide is covalently attached to a drug.

10. The pharmaceutical composition of claim 6, wherein said haptotactic peptide is covalently attached to the surface of a medical device.

11. The pharmaceutical composition of claim 10, wherein said haptotactic peptide is incorporated into a medical implant.

12. The composition of claim 11, wherein said haptotactic peptide is covalently attached to the surface of an implantable device.

13. The pharmaceutical composition of claim 12, wherein said haptotactic peptide is covalently attached to a bead.

14. The pharmaceutical composition of claim 12, wherein said haptotactic peptide is covalently attached to a matrix.

15. The pharmaceutical composition of claim 7, further comprising at least one additional haptotactic peptide.

16. The pharmaceutical composition of claim 7, further comprising a plurality of cells selected from the group consisting of mesenchymal cells,. parenchymal cells, fibroblasts, endothelial cells, chondrocytes, kidney cells, liver cells, pancreatic cells, thyroid cells, glial cells, astrocytes, smooth muscle cells and myofibroblasts.

17. The pharmaceutical composition of claim 7, further comprising a plurality of cells selected from the group consisting of immortalized cells, transformed cells, mammary carcinoma cells, 3T3 fibroblasts, malignant melanoma cells and ovarian carcinoma cells.

18. The pharmaceutical composition of claim 17, wherein said cell is selected from pluripotent cells capable of differentiating into fibroblasts, myofibroblasts, smooth muscle cells, endothelial cells, and combinations thereof.

19. The pharmaceutical composition of claim 17, wherein said cell type is selected from the group consisting of neural cells, glial cells, astrocytes, and combinations thereof.

20. The pharmaceutical composition of claim 17, wherein said cell type is selected from the group consisting of cells derived from bone marrow, blood or buffy-coat capable of differentiating into osteoblasts, chondrocytes, and combinations thereof.

21. The composition of claim 17, wherein said cell type is selected from the group consisting of immortalized cells and hybridomas.

22. A polymer composition, comprising:

a plurality of subunits, each of said subunits comprising at least one haptotactic peptide selected from the group consisting of SEQ ID NOs. 1-10 as well as analogues, derivatives, homologues and active fragments thereof; and further comprising a plurality of linker moieties for covalently linking a plurality of subunits to another to form the polymer.

23. An antibody capable of binding a haptotactic peptide, said peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:1-10.

24. An isolated polynucleotide encoding a haptotactic peptide selected from the group consisting of SEQ ID NOs.:1-10.

25. A diagnostic composition, comprising as an active ingredient a haptotactic peptide having a sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 SEQ ID NO. 12, and analogues, derivatives, fragments and homologues thereof.

26. The pharmaceutical composition claim 24 wherein the haptotactic peptide is derivatized with fluorescent tags.

27. The pharmaceutical composition of claim 24 wherein the haptotactic peptide is derivatized with radioactive tags.

28. The diagnostic composition of claim 24 wherein the haptotactic peptide is used to bind to fibrin clots for in vivo imaging and diagnosis of embolisms.

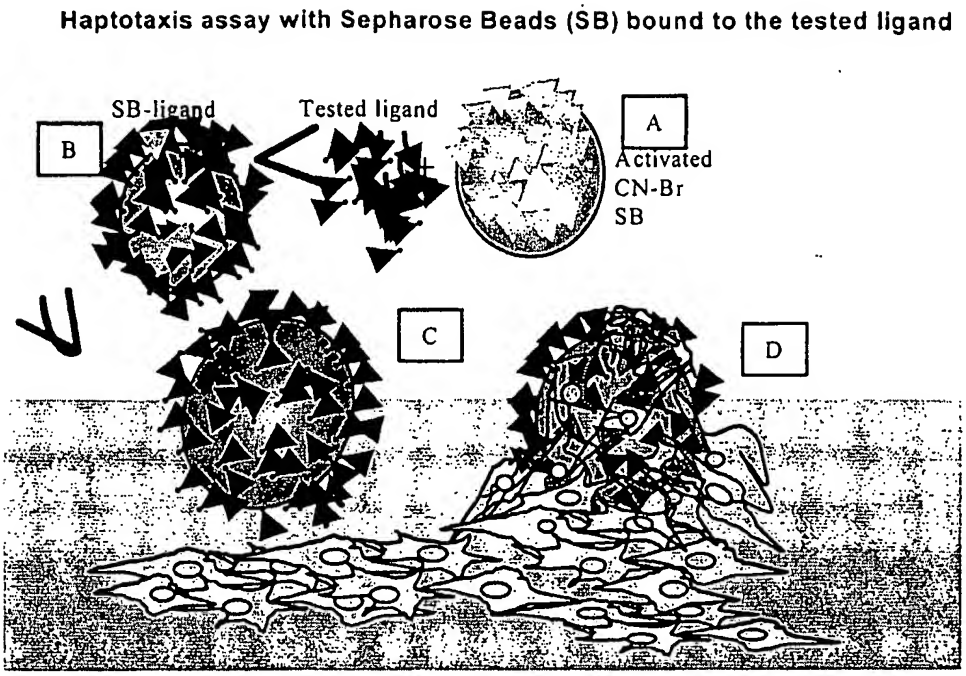
29. A method of treating a patient in need thereof with a haptotactic composition, said composition comprising a therapeutically effective amount of a haptotactic peptide having a sequence selected from the group consisting of SEQ ID NOs. 1-12 and analogues, derivatives, fragments and homologues thereof.

30. The method of claim 28 where said haptotactic peptide is useful to enhance wound healing.

31. The method of claim 28 where said haptotacitc peptide is useful to enhance osteogenesis.

32. The method of claim 28 where said haptotacitc peptide is useful to modulate angiogenesis in vivo.

Figure 1


Haptotaxis assay with Sepharose Beads (SB) bound to the tested ligand
SB-ligand
Tested ligand
A
Activated
CN-Br
SB
B
C
D

Haptotaxis of SB-ligand to BAEC

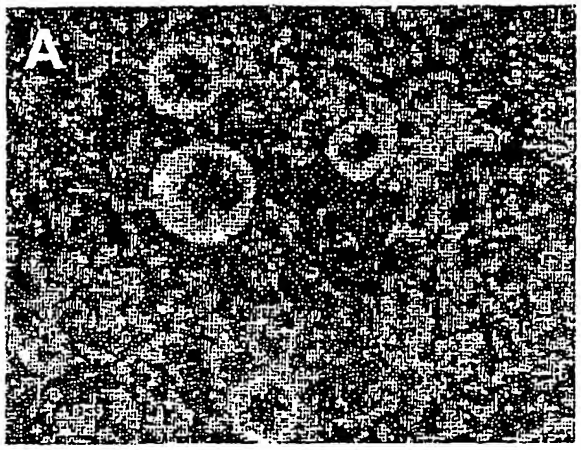


FIGURE 2A

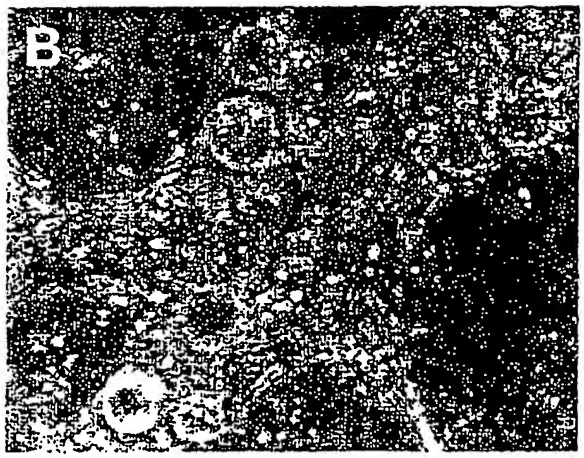


FIGURE 2B

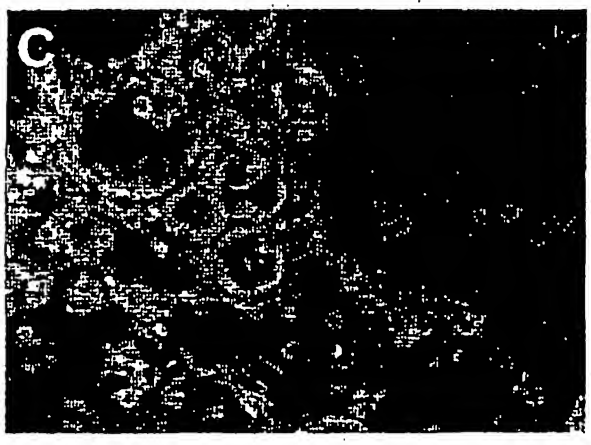
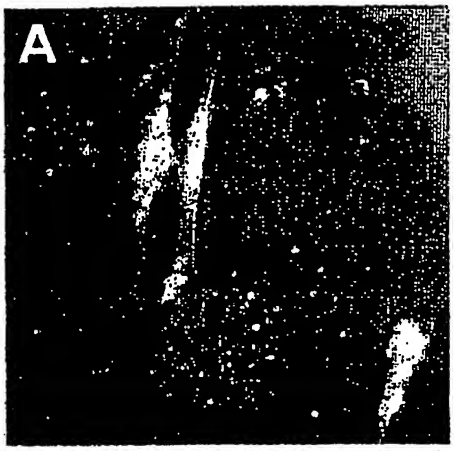


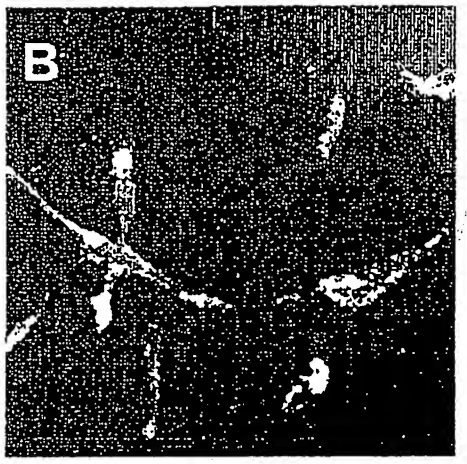
FIGURE 2C

HF Uptake of haptotactic peptide-FITC (1hr)



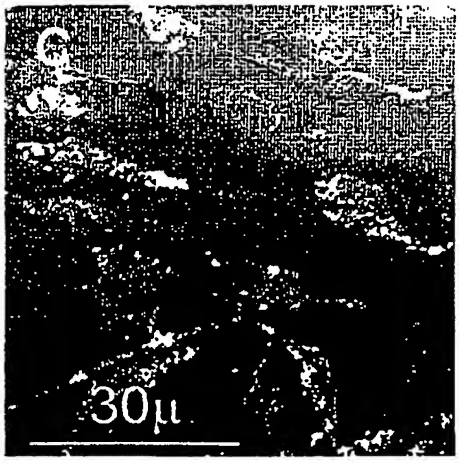
Cβ

FIGURE 3A



preCγ

FIGURE 3B



Cmfap

FIGURE 3C